STUDY OF ELETROSPINNING PCL/PLLA BLENDS

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Abstract. *Polymers are long-chain molecules that are formed by linking repetitive monomer units and they have been extensively studied for tissue-engineering applications. Structures that are designed as a support matrix to deliver cell populations or induce surrounding tissue growth are known as scaffolds. Biocompatible and biodegradable polymeric materials are considered as an important class of materials that can be used as scaffolds in tissue engineering applications. Scaffolds have to present similar structure and also function as an artificial extra-cellular matrix for cell attachment and growth. Some methods to fabricate scaffolds are solvent casting, fiber bonding, phase separation, gas induced foaming, salt leaching, 3D printing, selective laser sintering, multi-phase jet solidification, eletrospinning among others. The process of electrospinning is considered one of the most promising methods for the fabrication of polymer nanofibers. This process consists in applying a high electric field, which causes stretching of a polymer that exits through a capillary. Among the numerous applications of this process, electrospinning allows the fabrication of semiconductor and conductive nanofibers from mixtures or solutions, which have great potential for applications in sensors and the fabrication of scaffolds for cell growth. The poly (ε-caprolactone) (PCL) has been widely used for biomedical applications due to its biodegradable and biocompatible properties, and also presents the approved of U. S. Food and Drug Administration (FDA). PCL is a semi-crystalline aliphatic polymer that has a slow degradation rate around 12 – 24 month. It has glass transition temperature at – 60°C, a melting temperature at about 60°C. Poly-L-lactide (PLLA) is the product resulting from polymerization of L-lactide. PLLA has a degree of crystallization of approximately 37%, a glass transition temperature between 50–80°C and a melting temperature between 173–178 °C. Because of the stereo regular chain microstructure, optically pure polylactides, poly (L-lactide) (PLLA) and poly (D-lactide) (PDLA), are semi-crystalline. In this work the aim was to produce a blend of polycaprolactone and poly (L-lactide) using the method of eletrospinning for a production of scaffold. The blends were fabricated using poly (ε-caprolactone) (PCL, from Aldrich), with Mw of 80 000 g/mol, and poly (L-lactide) (PLLA, sintered in laboratory), with Mw of 240 000 g/mol, were dissolved in chloroform (CHCl3, Merck) and acetone (Synth) by stirring for 6 hours. The solution was electrospun for 1 hour using an equipment built by the research group, the voltage, flow rate and distance from the tip of the needle to the collector were 13 kV, 0.5ml/h and 12 cm, respectively. The morphology of the samples was observed by images obtained with scanning electron microscopy (SEM). Samples were also characterized by FT-IR and DSC.*

Keywords: *Polymers, Poly (ε-caprolactone), poly (L- Lactic Acid), electrospinning.*

1. INTRODUCTION

Tissue engineering can be defined as "'an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain or improve tissue function" (Langer and Vacanti, 1993).

Tissue-engineering strategies aim for mimicking the in vivo process of bone repair in a laboratory setting. The three key elements for generating bone tissue, namely osteogenic progenitor cells, osteoinductive growth factors and osteoconductive matrices are involved in this process.

Scaffolds are temporary matrices for bone growth and provide a specific environment and architecture for tissue development. The material composition as well as structural characteristics such as external and internal design is putatively crucial for the successful outcome of all scaffold-based bone tissue-engineering strategies.

In tissue engineering the materials for the cells support, called scaffolds are very important. It needs to offer a similar function as an artificial extra cellular matrix onto which cells attach, grow and form new tissues. Some methods to fabricate scaffolds are electrospinning, solvent casting, fiber bonding, phase separation, gas induced foaming, salt leaching, 3D printing, selective laser sintering, multi-phase jet solidification and others (Mikos, et al., 1996; Harris, et al., 1998; Hutmacher, et al., 2004; Patist, et al., 2004 and Pezzin, et al., 2002).

Electrospinning, has attracted a lot of attention due to its relative simplicity regarding the generation of fibrous scaffolds with nanoscale dimensions. Electrospinning utilizes electrostatic forces to spin polymer solutions or melts into whipped jets, producing continuous fibers with diameters from a few nanometers to micrometers after solvent evaporation in the spinning process (Pham, et. al, 2006; Yang, et al., 2009).

Polymers are organic or inorganic materials, whose structures are composed of repeating units, known as mere, which are linked by covalent bonds. Among the polymers that are used for more than two decades in the medical field, the poly (α-hydroxy acids) are highlighted, since they are considered as one of the most promising family of polymers in the area of bioresorbable materials. The form of degradation of these polymers occurs by hydrolysis of their ester bonds. Also, poly (lactic acid) (PLLA), poly (glycolic acid) (PGA) and poly (ε-caprolactone) (PCL) are substances that are approved by the Food and Drug Administration (FDA).

The poly (ε-caprolactone) (PCL) has been used in many researches because of its biodegradable, biocompatible properties and also has the approval of U. S. Food and Administration (FDA). PCL is a semi-crystalline aliphatic polymer that has a slow degradation rate 12 – 24 month. It has a low glass transition temperature at – 60°C, a melting temperature at about 60°C, and a high thermal stability (Cardoso, et al, 2010).

Poly (L-lactic acid) (PLLA) has been used in many researches because the characteristics of biocompatibility, degradation and absorption in aqueous medium. PLLA is a semicrystalline polymer with a melting point around 170 º C and a crystallinity around 70%. Among the poly (lactic), PLLA is the one which has the lowest rate of degradation.

By using only one type of polymer for the fabrication of scaffolds, all the necessary characteristics for a given clinical application is often not attained. Thus, research has been directed to study of polymer blends, copolymers and composites that improve the fundamental properties of scaffolds, such as permeability, absorption and elastic properties (Zhang et al., 1995).

PLLA/ PCL blends can be used to obtain improved properties regarding degradation rates, porosity and resistance to stress. Also, a critical characteristic is that they can be molded in different sizes and shapes. In this work the aim was to produce blends of polycaprolactone and poly (L-lactide) using the method of electrospinning for scaffold production. The blends were fabricated using poly (ε-caprolactone) (PCL, from Aldrich), with Mw of 80 000, and poly (L-lactide) (PLLA, sintered in laboratory).

2. MATERIALS

PLLA was synthesized by opening of cyclic dimer of lactic acid (lactide), with the objective of obtaining high molecular weight polymer (Motta et al., 2006).

The blend was made using poly (ε-caprolactone) (PCL, from Aldrich), with Mw of 80 000 g/mol, and PLLA, with Mw of 240 000 g/mol, was dissolved in chloroform ($CHCl_3$, Merck) and acetone [$(CH_3)_2CO$, Synth] with a rate of 25/75 wt%, respectively. The mixtures were stirring during 6 hours (Mikos et al., 1993).

The solution was electrospun for 1 hour using an equipment built by the research group, the voltage, flow rate and distance from the tip of the needle to the collector were 13 kV, 0.5ml/h and 12 cm, respectively. The different percentages of PCL/PLLA are shown in the Table 1.

Table 1: Different percentages of the blends.

Sample	PCL wt%	PLLA wt%
1	25	75
2	50	50
3	75	25

Instrumental characterization

The morphology of the samples was observed by images made with scanning electron microscopy (SEM) using the equipment Jeol (JXA 840 A). The fiber average diameter and standard deviation was obtained by the analysis of 8 fibers in the SEM images.

The samples were also analyzed by Fourier Transform Infrared Spectroscopy (FTIR) (4000 to 500 cm^{-1}). Transmission spectra of samples were obtained using THERMO SCIENTIFIC NICOLET IR100 spectrometer.

Differential scanning calorimetry (DSC) were used to obtain the thermal properties of the blends of PLLA / PCL using a METTLER TOLEDO DSC 823e. The samples were weighed into an aluminum sample port and hermetically sealed. The tests were performed under nitrogen atmosphere at 45mL/min and two scans temperature range:

- First scan of 0 ºC to 300 ºC at a rate of 10 ºC/min;
- Second scan of 0 ºC to 300 ºC at a rate of 10 ºC/min; interspersed with a cooling rate of 20 ºC/min.

3. RESULTS AND DISCUSSION

DSC analysis confirmed the blend of those polymers. For all the heating the peaks showed in the same temperature showing that the blends obtained are predominantly immiscible (Figure 1). The sample with more PCL content (red line) presents a more pronounced Tm peak due to PCL, when compared with the sample with more PLLA in the composition (blue line), which present an exothermic peak, which may be due to interaction of PLLA to PCL. The temperature of 175 ° C is next to the melting temperature of PLLA for the three curves.

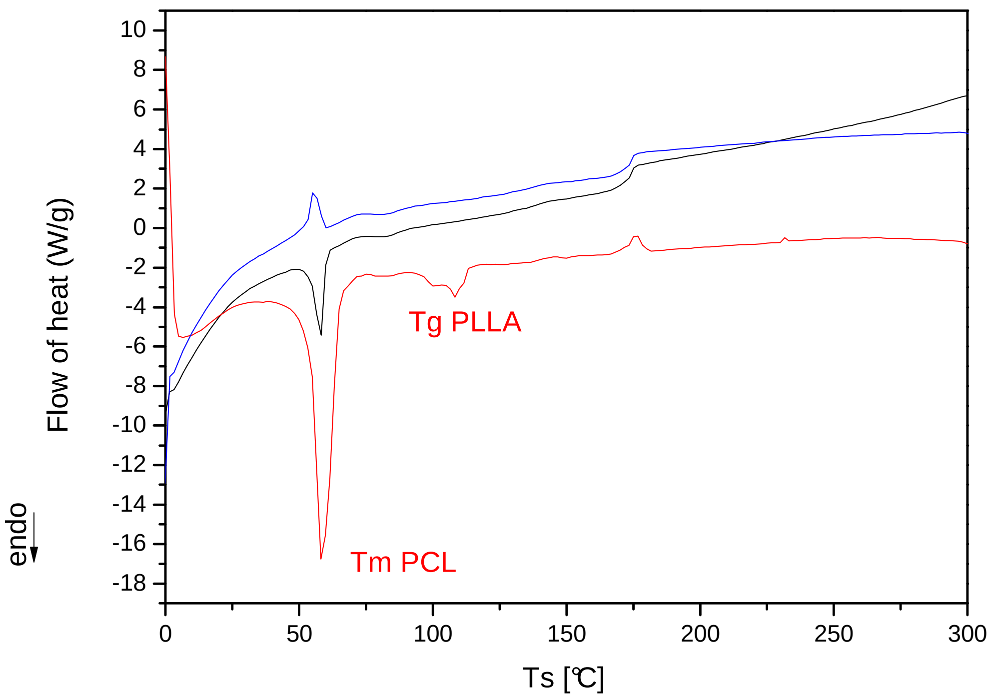

Figure 1: DSC of the samples: blue- 1; black-2 ; red- 3.

Figure 2 shows the spectrum of PLLA (green line). All samples were dislocated 300 cm^{-1}. Therefore the peak at 2995 cm-1 corresponds to alkane stretch (C–H). The C=O peak is at 1750 cm^{-1} while peak at 1187 cm^{-1} is for C–O group.

Figure 2 shows spectrum of PCL (red line). The O–H bond is at 3443 cm^{-1}. The peaks appearing at 2943 and 2866 cm^{-1} are due to the C–H stretching. The peak at 1724 cm^{-1} is due to the C=O bonding. The C–O bending is at 1167 cm^{-1}.

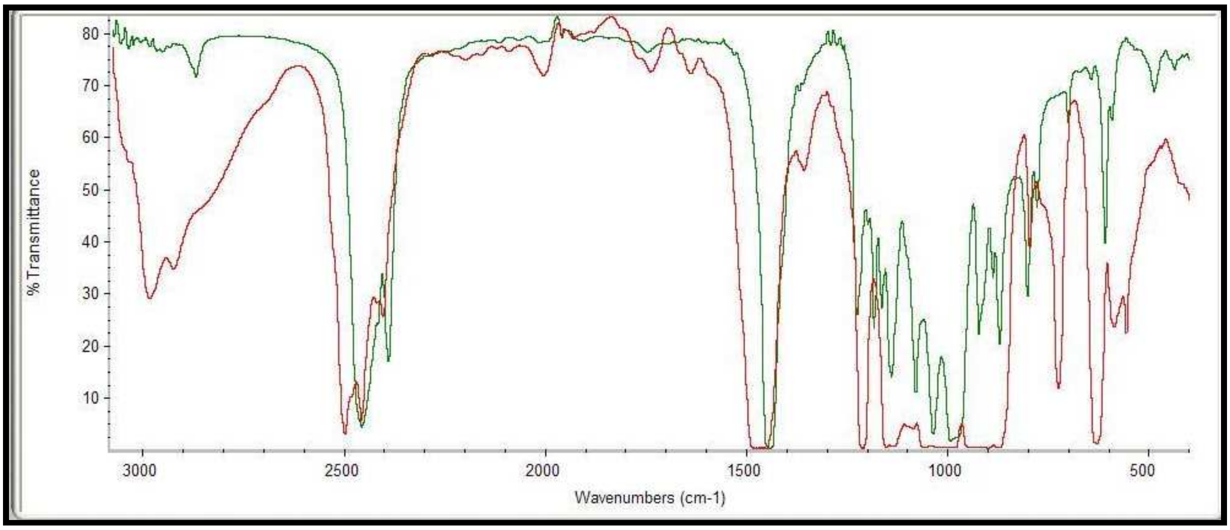

Figure 2: FT-IR green line- PLLA; red line- PCL.

Figure 3 shows the spectrum of the polymer blends (PLLA/PCL). The peak at 3358 cm^{-1} corresponds to O–H bond. The C–H stretching is at 2992 and 2945 cm^{-1}. The peak at 1747 cm^{-1} is due to the C=O bonding and at 1184 cm^{-1} corresponds to the C–O bending. These groups indicate the presence of both polymers (PLLA/PCL) in the blends.

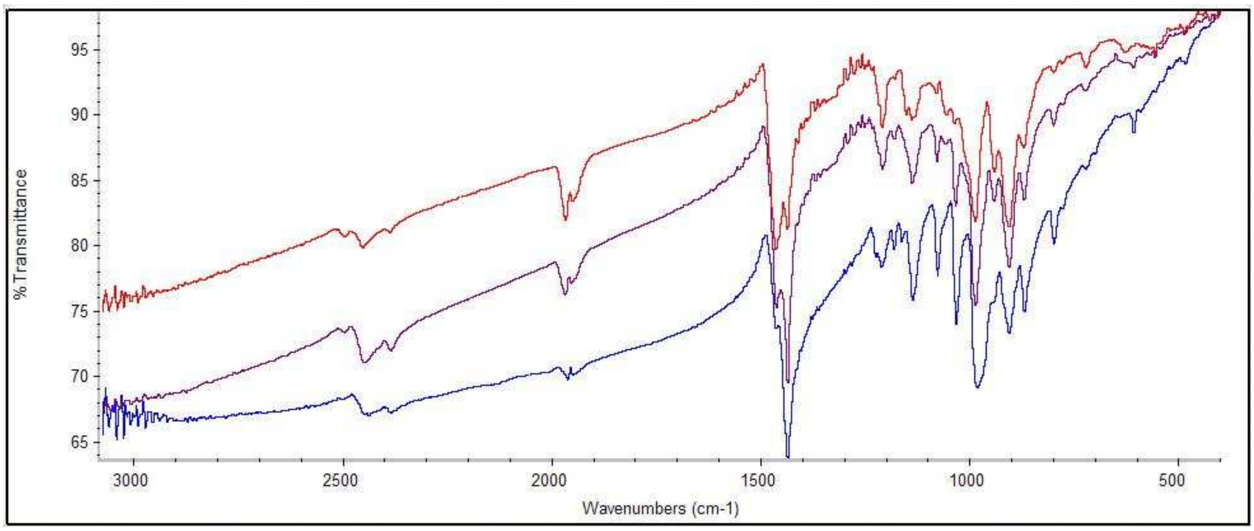

Figure 3: FT-IR red line- sample 1; purple line- sample 2; and blue line – simple 3.

The morphology of the different blends are shown in fig. 4. It can be seen for all samples that a nonwoven fibrous system with high porosity were obtained. It is clear the dependence of the fiber diameter with the blend composition. Table 2 presents the measured average diameter for samples 1,2 and 3. It can be seen that for sample 1, fibers with average diameter of 1,31 µm were obtained, which is close to the value obtained in sample 2, where the average diameter was 1,37 µm. However, the blend with equal proportion of PCL and PLLA, the average diameter presented a lower value of 0,79 µm.

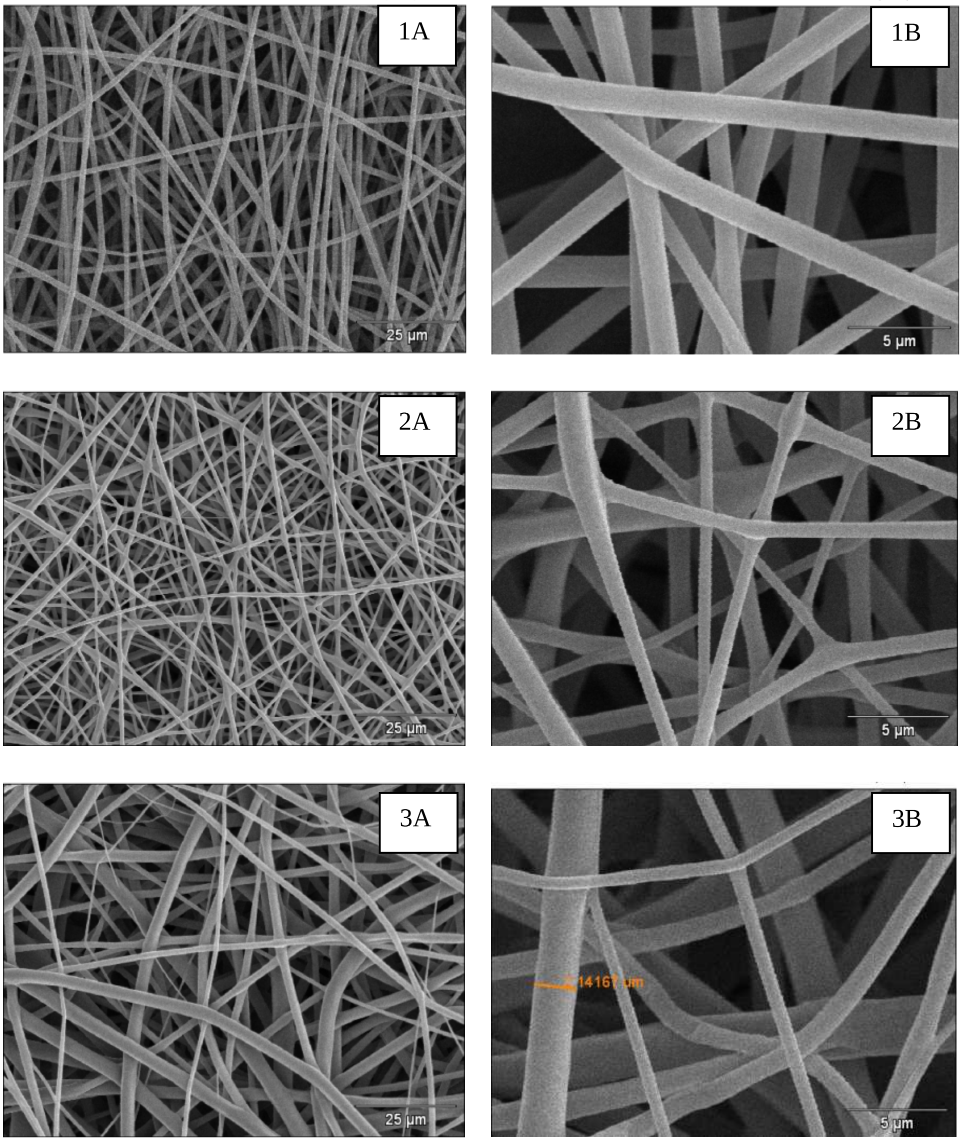

Figure 4: SEM images of (1) sample 1; (2) sample 2; (3) sample 3 in (A) 1000x and (B) 5000x.

Table 2: Average of the samples.

Samples	Average (µm)	Standard deviation (µm)
1	1.31	+/-0.13
2	0.79	+/-0.36
3	1.37	+/-0.83

The structural and functional properties of the natural extracellular matrix (ECM) are crucial for the proliferation, differentiation and migration of cells. As a consequence, there is an increasing tendency to design scaffold materials, as applied in tissue regeneration approaches, according to the characteristics of the ECM. The angle of contact can be very useful for the adhesion and proliferation of cells, therefore is very important to study this aspect, which will be the aim in further studies.

4. CONCLUSION

The use of biomaterials has been regarded as an efficient alternative to the allograft, since it significantly reduces the risk of rejection and local inflammation. Also, the method of preparation of the biopolymer together with it properties may influence in the degradation rate, flexibility and cell adhesion. Fibrous mats with high porosity of PCL/PLLA blends were successfully obtained by electrospinning, showing the potential use of electrospinning to fabricate scaffolds for tissue engineering. It was observed that the fiber morphology depends on blend composition.

5. REFERENCES

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